FINAL REPORT

ENTITLED:

" EVALUATION OF ANALYTICAL PROFILE INDEXING (API), FATTY ACID PROFILING ANALYSIS AND PULSED FIELD GEL ELECTROPHORESIS ANALYSIS OF *E. COLI* BACTERIA IN ENVIRONMENTAL SAMPLES TO IDENTIFY POLLUTION SOURCES"

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EXECUTIVE SUMMARY

Urbanization of upland areas adjacent to estuarine ecosystems has resulted in significant inputs of bacterial and chemical contaminants in salt marsh ecosystems of the southeastern US (Vernberg et al. 1992). During the pioneering stages of urban development, human waste disposal needs were met by use of septic tank based technology. As urban development proceeds and critical carrying capacity for human population density is reached, significant inputs of bacterial pollution from septic tank discharges into estuarine ecosystems may result (El-Figi 1991), often causing closure of shellfish harvesting waters due to the presence of pathogenic bacterial/viral pollution (Leonard 1992). The normal solution to this problem is to construct a central sewer collection system to reduce estuarine inputs from individual septic tank systems (Jolley 1978).

To address this problem of bacterial contamination from human waste associated with coastal urbanization, the Urbanization in Southeast Estuarine (Eco)Systems (USES) Study has evaluated the effects of human encroachment on estuarine surface waters quality and oyster quality/health. Two estuarine ecosystems were chosen for study: North Inlet (NI), a pristine estuary which is a National Estuarine Research Reserve site, and Murrells Inlet (MI), the most urbanized coastal area in the state of South Carolina (based upon population densities => 625/sq. mile). Results of this study indicated that a total of 67% of the surface water monitoring stations in MI exceeded the SA water quality criteria for fecal coliform bacteria (13/100ml) compared to only 33% of the stations in NI. These results indicated greater human sources of fecal coliform bacteria in urbanized MI.

Fecal coliform bacterial biotyping of surface waters indicated there were significant differences in the speciation of coliform positive species in surface waters of MI and NI. In urbanized MI, there was a greater occurrence of *E. coli* bacteria, fewer stations which were coliform negative and a reduced number of bacterial species comprising the coliform group, particularly soil sorbed microbes of the Pseudomonad family. Other findings from the USES Study indicated the results from surface water coliform biotyping indicated that there were greater potential risks of oyster exposure to *E. coli* and other pathogenic coliform members in urbanized MI than in pristine NI. Oyster results indicated that the greater potential human health risks measured in surface waters were not translated into greater actual or realized human health risks in terms of oyster bioconcentration potential. These results suggest that while there are clearly greater inputs of fecal coliform bacteria from human waste sources in urbanized areas, the process of tidal dilution and dispersion resulted in no discernible differences in oyster bioconcentration of these pathogens. Indeed the fecal coliform "finger prints" based upon oyster bioconcentration were not significantly different nor were there quantifiable differences in coliform densities in oysters between the two estuaries. These results clearly indicated there is a need to develop water quality analytical methods to discern fecal coliform bacterial sources, human versus wildlife.

Recent studies by Simons et al. (1995; 1996) clearly indicated two new potential

technologies, Pulsed Field Gel Electrophoresis and Fatty Acid Profiling, which have been used in other regions of the US to help discriminate human versus wildlife fecal coliform pollution sources. As a result of reviewing published data on these techniques the National Marine Fisheries Service and the Bureau of Ocean Resource Management at the South Carolina Department of Health and Environmental Control embarked on a collaborative research project to assess these new techniques in their ability to discriminate pollution sources in surface water samples.

These two new techniques, Pulsed Field Gel Electrophoresis (PFGE) and Fatty Acid Profiling (FAP), were used to discriminate animal versus human sources of *E. coli* bacteria using techniques and methods developed by Dr. George Simons at Virginia Tech University. Initially, laboratory cultures of *E. coli* biotype from different animal species (musk rat, raccoon, deer, otter and geese) and humans were tested by Analytical Profile Index (API) biotyping, PFGE and FAP to confirm earlier methods and results from Virginia Tech. Subsequent sampling of selected male and female human volunteers was conducted to examine E. coli variability in human fecal swabs. Final sampling of a septic tank at the International Center for Public Health Research at the Wedge Plantation was conducted along with samples from a watershed in North Inlet dominated by inputs from wildlife and birds were sampled.

Results to date indicated that API profiling was useful in obtaining pure *E. coli* biotype cultures from animals and human samples but that no one API biotype for *E. coli* was specific for human or wildlife samples. FAP analysis used extraction methods to saponify and extract fatty acids from E. Coli samples as fatty acid methyl ethers (FAME). Gas Chromatography-Mass Spectroscopy (GCMS) results identified 21 fatty acids (C12 to C20) in *E coli* samples which accounted for 95% of the total fatty acid components. Two unidentified fatty acid components were found in all samples which accounted for < 1% of the total fatty acid components. FAP was able to discriminate between human and wildlife samples some 96.2% of the time, using principal component analysis for two selected lipids which accounted for 44% of the total variance in these data. Principal component analysis of FAP was not able to discriminate between different wildlife species, thus we propose to include all wildlife samples as a class when comparing with human isolates. FAP analysis of human *E coli* isolates grown on broth versus plate media, indicated that culture media greatly influenced FAP results, specifically for the 19:0 cyc lipid fraction (9.78-12.55% in broth versus 4.44-7.07% in plate culture). This indicates that comparison of results from our study with other literature sources involving other bacterial culture methods may be difficult, since culture conditions may greatly affect results. PFGE results indicated that when *E. coli* sources could be identified (human versus wildlife= 60% of the time), there was a 90% probability in discriminating wildlife versus human sources. PFGE analysis further confirmed that the *E coli* biotypes found within an individual human stool sample were unique to that individual. All (100%) male samples contained *E. coli* (biotype codes 7144552 and 7144572). Only 33.3% of the female samples contained *E. coli* (biotype code 1044552) along with *Klebsiella pneumoniae* and *Enterobacter sakazakii*. The PFGE and FAP methods have the potential ability to discriminate between human and wildlife sources of *E. coli*. Further validation of PFGE and FAP is on going in septic tank and field samples. This information would be invaluable to environmental managers to better manage these impacts from urbanization.

INTRODUCTION

Urbanization of upland areas adjacent to estuarine ecosystems has resulted in significant inputs of bacterial and chemical contaminants in salt marsh ecosystems of the southeastern US (Vernberg et al. 1992). During the pioneering stages of urban development, human waste disposal needs were met by use of septic tank based technology. As urban development proceeds and critical carrying capacity for human population density is reached, significant inputs of bacterial pollution from septic tank discharges into estuarine ecosystems may result (El-Figi 1990), often causing closure of shellfish harvesting waters due to the presence of pathogenic bacterial/viral pollution (Leonard 1993). The normal solution to this problem is to construct a central sewer collection system to reduce estuarine inputs from individual septic tank systems (Jolley 1978).

To address this problem of bacterial contamination from human waste associated with coastal urbanization, the Urbanization in Southeast Estuarine (Eco)Systems (USES) Study has evaluated the effects of human encroachment on estuarine surface waters quality and oyster quality/health. Two estuarine ecosystems were chosen for study: North Inlet (NI), a pristine estuary which is a National Estuarine Research Reserve site, and Murrells Inlet (MI), the most urbanized coastal area in the state of South Carolina (based upon population densities => 625/sq. mile).

Results of this study indicated that a total of 67% of the surface water monitoring stations in MI exceeded the SA water quality criteria for fecal coliform bacteria (13/100ml) compared to only 33% of the stations in NI. Poor water quality stations in MI were associated with high densities of septic tanks in close proximity to the estuary and other urban activities (marinas, boat landings and roadways). GIS overlays and statistical analysis indicated that regions in MI with high levels of PAHs, near roadways and marinas, also had concomitant high fecal coliform bacteria densities. This suggest that fecal coliform bacterial densities may be affected (due to biostimulation) in areas with high PAH concentrations. Poor water quality in NI was associated with upland areas housing large populations of birds and wildlife. These findings clearly indicate that fecal coliform bacteria pollution is associated with urbanization and that closure of shellfish harvesting waters may be perhaps the most significant, quantifiable impact from urbanization.

Fecal coliform bacterial biotyping of surface waters indicated there were significant differences in the speciation of coliform positive species in surface waters of MI and NI. In urbanized MI, there was a greater occurrence of *E. coli* bacteria, fewer stations which were coliform negative and a reduced number of bacterial species comprising the coliform group, particularly soil sorbed microbes of the Pseudomonid family. In pristine NI, surface waters had a greater number of coliform negative stations, a reduced occurrence of *E. coli* bacteria and an increased number of bacterial species comprising the coliform group with an increased occurrence of soil-sorbed microbes in the Pseudomonad family. The greater diversity/species richness in the coliform group members in NI resulted from the availability of bacteria from the deciduous hardwood forest when compared to upland watersheds in urbanized MI, which contain

more monoculture (i.e. lawns with grass and ornamental plants) habitat.

Fecal coliform bacterial biotyping of oysters indicated that unlike results for surface waters, there were no significant differences in the speciation of coliform positive species in oysters from MI and NI. One factor related to this observation may have been that the high levels of fecal coliform bacteria which were measured at ebb tide, may have been diluted significantly at flood tide (when oyster feed) to comparable densities, which were then bioconcentrated equivalently by oysters in each estuary.

As these findings from the USES Study indicated the results from surface water coliform biotyping indicated that there were greater potential risks of oyster exposure to *E. coli* and other pathogenic coliform members in urbanized MI than in pristine NI. Oyster results indicated that the greater potential human health risks measured in surface waters were not translated into greater actual or realized human health risks in terms of oyster bioconcentration potential. These results suggest that while there are clearly greater inputs of fecal coliform bacteria from human waste sources in urbanized areas, the process of tidal dilution and dispersion resulted in no discernible differences in oyster bioconcentration of these pathogens. Indeed the fecal coliform "finger prints" based upon oyster bioconcentration were not significantly different nor were there quantifiable differences in coliform densities in oysters between the two estuaries. ***This suggests that the current Interstate Shellfish Sanitation Conference (ISSC) method of regulating shellfish harvesting which is based on surface water quality provides a margin of safety but may be some what over protective, as there were no actual differences in fecal coliform levels in oysters between the two estuaries.*** With recent international agreements reached on trade (i.e. NAFTA and GATT) there may be increased pressures for the U.S. to adopt a new policy on oyster meat standards in addition to our current shellfish harvesting, surface waters standard.

As these results clearly indicated there is a need to develop water quality analytical methods to discern fecal coliform bacterial sources, human versus wildlife. Recent studies by Simons et al. (1995; 1996) clearly indicate two new potential technologies, Pulsed Field Gel Electrophoresis and Fatty Acid Profiling, which have been used in other regions of the US to help discriminate human versus wildlife fecal coliform pollution sources. As a result of reviewing published data on these techniques the National Marine Fisheries Service and the Bureau of Ocean Resource Management at the South Carolina Department of Health and Environmental Control embarked on a collaborative research project to assess these new techniques in their ability to discriminate pollution sources in surface water samples.

These two new techniques, Pulsed Field Gel Electrophoresis (PFGE) and Fatty Acid Profiling (FAP), were used to discriminate animal versus human sources of *E. coli* bacteria using techniques and methods developed by Dr. George Simons at Virginia Tech University. Initially, laboratory cultures of *E. coli* biotype from different animal species (musk rat, raccoon, deer, otter and geese) and humans were tested by Analytical Profile Index (API) biotyping, PFGE and FAP to confirm earlier methods and results from Virginia Tech. Subsequent sampling of selected male and female human volunteers was conducted to examine *E. coli* variability in human fecal swabs. Final sampling of a septic tank at the International Center for Public Health Research at the Wedge Plantation was conducted along with samples from a watershed in North Inlet dominated

by inputs from wildlife and birds were sampled.

STUDY DESIGN

This study was focused on three major research techniques: Analytical Profile Indexing (API), Fatty Acid Profiling (FAP) and Pulsed Field Gel Electrophoresis (PFGE). The methods used for each for each technique are presented in detail along with results within each chapter of the report. The overall analytical scheme for API, FAP and PFGE methodologies was to:

1. Analyze laboratory cultures of E. Coli isolated from human and wildlife (musk rat, deer, otter, goose and raccoon) samples;

2. Analyze rectal swabs from male and female human volunteers:

3. Analyze surface water samples from a septic tank and a water shed dominated by wildlife input: and

4. Comparison of PFGE results from the NMFS with results from Virginia Tech.

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"ANALYTICAL PROFILE INDEX ANALYSIS OF SELECTED ENVIRONMENTAL SAMPLES FOR *E. COLI*"

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Fecal Coliform Study

Six *E. coli* isolates from 5 different animal species, as well as 6 isolates from humans were received from George Simmons. Six samples from humans (3 male and 3 female) and one raccoon from the Charleston area have also been collected. One isolate from each of the above species was transferred to lauryl tryptose broth (LTB) and incubated for 24 hrs. After 24 hrs, positive LTB tubes (indicated by gas production in the tubes) were transferred to EC Broth which is specific for *E. coli*. After 24 hrs, the positive EC tubes (indicated by gas production) were streaked for isolation onto violet red bile agar (VRBA). These plates were incubated for 24 hrs. A colony from the VRBA plates was streaked for isolation on plate count agar (PCA) plates. After a 24 hr incubation, a colony was picked from the PCA plate, placed into 5 ml of 0.85% saline, and vortexed. Then, Analytical Profile Index (API) tests were run according to the prescribed protocol.

So far, API's have been run on the following:

(1) isolate from each species received from George Simmons (human, deer, otter, muskrat, goose, and raccoon);

(3) isolates from a local raccoon (Jan's raccoon);

(5) isolates from 5 humans in the Charleston area.

Results

--- The 6 George Simmons isolates that have been run at NMFS thus far were confirmed as *E. coli* using API.

--- A comparison of George Simmons API codes to NMFS API codes shows that almost all NMFS codes had different first numbers with the exception of the human isolate.

--- The 3 isolates from Jan's Raccoon (Charleston area) were confirmed *E. coli* using API.

--- The API test results for the bacteria isolated from humans in the Charleston area are as follows:

---	Male:	#1	5 isolates with identical *E. coli* API codes (7144552);
		#2	5 isolates with identical *E. coli* API codes (7144572);
		#3	5 isolates with identical *E. coli* API codes (7144552);
---	Female:	#4	All 5 isolates with identical *Enterobacter sakazakii*

		API codes (3205373);
---	#5	No Growth in the LTB;
---	#6	4 isolates with identical *E. coli* API codes (1044552); 1 isolate was identified as *K. pneumoniae* (API code 7215773).

Discussion

The process of using non-selective and selective media to isolate *E. coli* has been useful in obtaining pure cultures of the bacteria from samples. The API test can be used to identify *E. coli*, and for this reason, the test has been useful in the project. However, the API will probably not be useful in identifying the specific source of the coliforms. Only a few animal species have been run thus far, but some have the same API codes as the human samples. In the near future, more API's need to be run on the other cultures from George Simmons to see how many animal codes match human codes.

NMFS FECAL COLIFORM STUDY - API CODES

CLAMS API #	# of ISOLATES		ANIMAL	LIBRARY #	SIMMONS API #	NMFS API #		
***5144572	34		Human	226	7144552	7144572		
**5144552	15		Jan's Raccoon #1			7144572		
*5044552	14		Jan's Raccoon #2			7144572		
5044572	7		Jan's Raccoon #3			7144572		
1144572	3		Raccoon	1	5144572	7144552		
1044412	3		Otter	107	5044552	7044552		
7144572	3		Goose	122	5144572	4144572		
1044552	2		Muskrat	88	5044572	7044572		
1044572	2		Deer	62	5144552	7144552		
1044402	2							
1044562	2		Human	Hu 1A		7144552		
5144562	2		Human	Hu 1B		7144552		
5144570	2		Human	Hu 1C		7144552		
5044573	1		Human	Hu 1D		7144552		
5144532	1		Human	Hu 1E		7144552		
5144512	1		Human	Hu 2A		7144572		
5044512	1		Human	Hu 2B		7144572		
5044553	1		Human	Hu 2C		7144572		
1044172	1		Human	Hu 2D		7144572		
			Human	Hu 2E		7144572		
TOTAL	98		Human	Hu 3A		7144552		
			Human	Hu 3B		7144552		
			Human	Hu 3C		7144552		
			Human	Hu 3D		7144552		
			Human	Hu 3E		7144552		
			Human	Hu 4A		3205373	Ent. Sakazakii	
			Human	Hu 4B		3205373	Ent. Sakazakii	
			Human	Hu 4C		3205373	Ent. Sakazakii	
			Human	Hu 4D		3205373	Ent. Sakazakii	
			Human	Hu 4E		3205373	Ent. Sakazakii	
			Human	Hu 6A		7215773	K. pneumoniae	
			Human	Hu 6B		1044552		
			Human	Hu 6C		1044552		
			Human	Hu 6D		1044552		
			Human	Hu 6E		1044552		
			Human					
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" FATTY ACID PROFILING (FAP) OF SELECTED ENVIRONMENTAL *E. COLI SAMPLES TO DISTINGUISH HUMAN VERSUS WILDLIFE SOURCES"*

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E. coli Project - Lipid Chemistry

Objective:
To determine if fatty acid profiling has potential for the differentiation of human/animal fecal coliforms.

Background:
Dr. George Simmons (Virginia Tech, Blacksburg, VA,), using fatty acid compositional analysis in conjunction with Principal Component Analysis (PCA) of human/animal fecal coliforms collected from a variety of host species, showed between species differences suggesting that fatty acid profiling could potentially be used to identify the source of fecal coliform contamination in environmental samples (Simmons, personal communication). This work was done using the MIDI system (MIDI, Inc., Newark, DE). This automated system, extensively used for species identification of bacteria, provides a software package that controls gas chromatograph (GC) operation, component identification, data reduction, multivariate analysis, database capability and pattern recognition database search program. Simmons' established database contained results from greater than 225 analyses, including a variety of species. Due to personnel changes at the university, this database was lost and the MIDI system is no longer available to him. Since this raw data and database is no longer available, new data must be acquired in order to evaluate the use this approach for identification of host species for fecal coliforms.

Approach:
Since we do not have the MIDI software package, essentially each segment of the process was incorporated by independently applying lipid analytical procedures currently used for each segment of the operation. Published methods for sample preparation were evaluated, and an appropriate method adapted. Published methods for gas chromatographic analysis of derivatized samples were evaluated, and a method adapted that produced acceptable results and compatibility with ongoing Charleston projects/analyses. Using selected methods, authentic samples received from George Simmons (multiple individuals from each of 6 species) were analyzed. These data were analyzed using Principle Component Analysis (SAS Institute, Inc., 1990).

Methods:
Culture: Cells from authentic samples (6 individual each) of human, deer, racoon, otter, goose and muskrat *E. coli* were cultured in broth as described elsewhere (see Thompson). Cells from human samples were also cultured on plates (duplicated on different days) as described elsewhere (see Thompson).

Sample preparation: Three published methods (Sasser, 1990; Miller, 1984; Moore et al., 1994) for sample preparation were evaluated using an in house reference triacylglycerol. Since bacterial fatty acid are found as membrane lipid components rather than as stored triacylglycerols, this was not a ideal reference material for this study, but was readily available, and should provide a reasonable comparison. The method that produced best recovery and agreement with known composition of the reference material was a modified method using elements from each of the three methods. Briefly, cells, as a pellet from broth or scraped from plates, were transferred to a 16 x 125 mm screw-cap culture tube. Saponification of the lipids was achieved by the addition of a strong base to the whole cells and heating. After cooling, the mixture was acidified by the addition of 6M HCl, 2 ml of BCl_3 (12% in methanol) was added, and the mixture heated at 85°C for 5 mins yielding fatty acid methyl esters (FAMEs). The FAMEs were extracted into an organic solvent, washed with an aqueous basic solution and transferred to vials for GC analysis.

Gas chromatographic analysis: FAMEs were analyzed by gas chromatography with separation achieved on a non-polar fused silica capillary column (dimethyl polysiloxane). A bacterial FAME standard was chromatographed as the first and last run of the batch to assist in identification of sample components and to monitor instrument performance. Individual fatty acids were tentatively identified by comparison of their relative retention times with those of authentic standards. Selected samples were analyzed by GC/MS to confirm identifications for major components. Amounts of individual fatty acids are reported as an area percent of total fatty acids.

Data analysis: Although only limited data were available, the data set was analyzed using Principal Component Analysis (PCA). The data set consists of 23 fatty acids (the 21 identified fatty acids and 2 unknown components) found in the profiles of individuals (31) from the six species.

Results: Twenty-one fatty acids (accounting for approximately 95% of the total fatty acids) were identified in the samples. Two components, found in all samples at < 1%, were not identified and are reported as uk1 and uk2. All samples, regardless of species, contained the same fatty acids in similar proportions. This was not unexpected since fatty acid profiling has been shown to be an effective method for identification of bacterial species. Minimum, maximum and average values for individuals for each species are shown in Tables 1-6. A summary of average values is given in Table 7. Prominent profile differences for the various host species were not apparent by visual inspection of the data. It has been demonstrated that multivariate statistics, such as PCA, enhances the interpretation of tables of fatty acid compositions (Grahl-Nielsen and Mjaavatten, 1991). Principal Component (PC)1 And PC2, describing the largest and second largest variance among the samples, accounted for 44% of the

variance. The plot of PC1 and PC2 is shown Figure 1. The samples from non-human did not show distinct grouping for individual species, but collectively constituted a group that included 25 of the 26 non-human samples, and was separated from the human sample group. However, there was substantial variation within the two groups. A single racoon sample, fell to the far right of the plot and within the human group. No explanation for this occurrence can be given at this time.

Due the equipment break-down (centrifuge), these analyses have not been replicated to determine repeatability for samples cultured on different days, nor was there sufficient sample to test repeatability for sample preparation and analysis. However, human *E. coli* samples were cultured on plates on two different days, and analyzed for fatty acid composition. Percentages of the major fatty acids were, in most cases, with $\pm$ 20% for the two analyses. Differences in the percentages of specific fatty acids were noted for samples cultured on plates as compared to those cultured in broth. Specifically, the percentage of 19:0cyc ranged from 9.78 to 12.55 (Table 1) for samples cultured in broth while those from plate cultures ranged from 4.44 to 7.07. The results from analysis of samples from plate cultures suggests that fatty acids compositions are reproducible for cells cultured under the same conditions, but will vary with culture protocols. This is in agreement with work done by other investigators (Sasser, 1990; Miller, 1984; Moore et al., 1994).

Although results from analyses of individual samples were not available from Simmons' analyses, summary information was provided that included the range of percentages found for each fatty acid for each species, as well as averages and standard deviations. This information was compared with that obtained for the Charleston analyses of samples cultured under similar conditions in broth (Table 8). Since two major components of the Simmons fatty acid profiles were not identified, nor was information available on GC analyses, direct comparison of total composition could not be made. However, differences were noted in the percentages of several of the fatty acids that were identified. This is likely due to differences in culture procedures and in sample preparation. Despite that fact that differences were noted between laboratories, results were consistent within a laboratory.

Results from PCA of the limited data set suggests that fatty acid profiling may have potential for identification of *E. coli* host species. Clearly, a larger sample set must be analyzed to determine whether or not the trend observed in this data set is consistent, and, if so, the confidence level that can be placed on this method of identification.

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TABLE 1. RANGE AND AVERAGE VALUES (AREA PERCENT) FOR FAME OF *E. coli* FROM HUMAN SAMPLES.

Fatty acid	226	227	228	229	231	*min*	*max*	**avg**	SD	RSD
C12:0	2.87	2.76	3.01	2.60	2.53	*2.53*	*3.01*	**2.75**	0.20	0.07
C13:0	0.14	0.15	0.14	0.17	0.09	*0.09*	*0.17*	**0.14**	0.03	0.21
3-OH 12:0	0.03	0.04	0.05	0.04	0.04	*0.03*	*0.05*	**0.04**	0.01	0.20
C14:1	0.50	0.48	0.53	0.38	0.38	*0.38*	*0.53*	**0.45**	0.07	0.15
C14:0	6.79	6.62	7.57	7.62	7.63	*6.62*	*7.63*	**7.24**	0.50	0.07
uk1	0.29	0.34	0.32	0.76	0.74	*0.29*	*0.76*	**0.49**	0.24	0.49
ai 15:0	0.05	<0.01	0.05	0.06	0.03		*0.06*	**0.04**	0.02	0.63
C15:0	0.91	0.86	0.96	1.11	0.61	*0.61*	*1.11*	**0.89**	0.18	0.21
2-OH 14:0	0.14	0.22	0.16	0.26	0.21	*0.14*	*0.26*	**0.20**	0.05	0.25
3-OH 14:0	5.07	5.06	5.29	5.76	5.86	*5.06*	*5.86*	**5.41**	0.38	0.07
16:1W7	2.80	3.26	4.28	4.44	4.92	*2.80*	*4.92*	**3.94**	0.88	0.22
C16:0	32.82	33.40	29.69	29.91	31.14	*29.89*	*33.40*	**31.43**	1.62	0.05
iso 17:0	<0.01	<0.01	<0.01	<0.01	0.11	*<0.01*	*0.11*	**0.02**	0.05	2.24
C17:1	0.06	0.09	<0.01	<0.01	0.18	*<0.01*	*0.18*	**0.07**	0.08	1.14
17:0 cyc	25.03	22.27	20.38	17.82	17.99	*17.82*	*25.03*	**20.70**	3.04	0.15
C17:0	0.43	0.47	0.45	0.53	0.27	*0.27*	*0.53*	**0.43**	0.10	0.23
2-OH 16:0	<0.01	<0.01	<0.01	<0.01	0.07	*<0.01*	*0.07*	**0.01**	0.03	2.24
uk2	0.43	0.25	0.22	0.54	0.51	*0.22*	*0.54*	**0.39**	0.15	0.38
18:1w7	4.95	6.12	10.93	7.90	7.83	*4.95*	*10.93*	**7.54**	2.26	0.30
C18:0	0.55	0.58	0.55	0.59	0.60	*0.55*	*0.60*	**0.57**	0.03	0.04
19:0 cyc	12.55	11.94	12.15	11.31	9.78	*9.78*	*12.55*	**11.55**	1.09	0.09
C19:0	<0.01	<0.01	<0.01	0.20	0.25	*<0.01*	*0.25*	**0.09**	0.12	1.38
C20:0	<0.01	<0.01	<0.01	<0.01	<0.01	*<0.01*	*<0.01*			

TABLE 2. RANGE AND AVERAGE VALUES (AREA PERCENT) FOR FAME OF *E. coli* FROM RACOON SAMPLES.

Fatty acid	racoon-1	racoon-2	racoon-4	racoon-5	racoon-6	*MIN*	*MAX*	**AVG**	SD	RSD
C12:0	2.82	2.85	2.54	3.22	3.25	*2.54*	*3.25*	**2.94**	0.30	0.10
C13:0	0.10	0.17	0.05	0.09	0.07	*0.05*	*0.17*	**0.10**	0.05	0.49
3-OH 12:0	<0.01	0.05	<0.01	0.03	0.03	*<0.01*	*0.05*	**0.02**	0.02	0.97
C14:1	0.46	0.46	0.42	0.39	0.43	*0.39*	*0.46*	**0.43**	0.03	0.07
C14:0	8.35	6.60	6.19	5.54	5.52	*5.52*	*8.35*	**6.44**	1.16	0.18
uk1	0.36	0.32	0.62	0.71	0.69	*0.32*	*0.71*	**0.54**	0.19	0.35
ai 15:0	<0.01	0.03	<0.01	<0.01	<0.01	*<0.01*	*0.03*	**0.01**	0.01	2.24
C15:0	0.49	0.85	0.37	0.43	0.42	*0.37*	*0.85*	**0.51**	0.19	0.38
2-OH 14:0	0.19	0.17	0.24	0.28	0.23	*0.17*	*0.28*	**0.22**	0.04	0.20
3-OH 14:0	5.14	5.05	5.34	6.22	6.21	*5.05*	*6.22*	**5.59**	0.58	0.10
16:1w7	3.25	5.51	3.32	4.09	4.09	*3.25*	*5.51*	**4.05**	0.91	0.22
C16:0	31.93	32.96	29.15	30.91	30.63	*29.15*	*32.96*	**31.11**	1.43	0.05
iso 17:0	<0.01	<0.01	<0.01	<0.01	<0.01	*<0.01*	*<0.01*			
C17:1	0.04	0.11	<0.01	<0.01	<0.01	*<0.01*	*0.11*	**0.03**	0.05	1.63
17:0 cyc	22.36	22.70	19.96	21.87	22.17	*19.96*	*22.70*	**21.81**	1.08	0.05
C17:0	0.26	0.38	0.22	0.29	0.27	*0.22*	*0.38*	**0.28**	0.06	0.22
2-OH 16:0	<0.01	<0.01	<0.01	<0.01	0.13	*<0.01*	*0.13*	**0.03**	0.06	2.24
uk2	0.45	0.47	0.33	0.45	0.40	*0.33*	*0.47*	**0.42**	0.06	0.13
18:1w7	4.79	7.63	5.36	6.93	6.68	*4.79*	*7.63*	**6.28**	1.17	0.19
C18:0	0.51	0.43	0.56	0.59	0.57	*0.43*	*0.59*	**0.53**	0.07	0.13
19:0 cyc	13.32	10.68	13.39	14.73	14.26	*10.68*	*14.73*	**13.28**	1.57	0.12
C19:0	<0.01	<0.01	<0.01	<0.01	0.22	*<0.01*	*0.22*	**0.04**	0.10	2.24
C20:0	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01			

TABLE 3. RANGE AND AVERAGE VALUES (AREA PERCENT) FOR FAME OF *E. coli* FROM DEER SAMPLES.

Fatty acid	deer-63	deer-64	deer-65	deer-66	deer-67	*MIN*	*MAX*	AVG	SD	RSD
C12:0	2.95	3.11	3.05	3.11	2.63	*2.63*	*3.11*	**2.97**	0.20	0.07
C13:0	0.05	0.09	0.08	0.07	0.07	*0.05*	*0.09*	**0.07**	0.01	0.20
3-OH 12:0	0.04	0.03	0.03	0.03	<0.01	*<0.01*	*0.04*	**0.03**	0.02	0.59
C14:1	0.36	0.37	0.40	0.42	0.42	*0.36*	*0.42*	**0.39**	0.03	0.07
C14:0	7.88	7.25	5.69	7.63	7.70	*5.69*	*7.88*	**7.23**	0.89	0.12
uk1	0.82	0.84	0.75	0.84	0.85	*0.75*	*0.85*	**0.82**	0.04	0.05
ai 15:0	<0.01	0.02	0.02	<0.01	<0.01	*<0.01*	*0.02*	**0.01**	0.01	1.37
C15:0	0.39	0.55	0.50	0.47	0.43	*0.39*	*0.55*	**0.47**	0.06	0.13
2-OH 14:0	0.18	0.19	0.02	0.21	0.22	*0.02*	*0.22*	**0.16**	0.08	0.50
3-OH 14:0	6.10	5.89	6.08	6.24	6.32	*5.89*	*6.32*	**6.13**	0.17	0.03
16:1W7	3.11	4.35	5.32	3.87	4.46	*3.11*	*5.32*	**4.22**	0.81	0.19
C16:0	30.32	29.89	30.81	30.24	30.44	*29.89*	*30.81*	**30.34**	0.33	0.01
iso 17:0	0.08	<0.01	0.07	<0.01	<0.01	*<0.01*	*0.08*	**0.03**	0.04	1.38
C17:1	<0.01	0.11	<0.01	<0.01	0.07	*<0.01*	*0.11*	**0.04**	0.05	1.42
17:0 cyc	20.08	20.72	20.73	19.68	20.53	*19.68*	*20.73*	**20.35**	0.46	0.02
C17:0	0.16	0.26	0.24	0.27	0.26	*0.16*	*0.27*	**0.24**	0.04	0.18
2-OH 16:0	0.06	0.07	0.06	0.06	<0.01	*<0.01*	*0.07*	**0.05**	0.03	0.57
uk2	0.49	0.42	0.38	0.47	0.46	*0.38*	*0.49*	**0.44**	0.04	0.10
18:1w7	5.17	7.99	7.60	6.85	7.85	*5.17*	*7.99*	**7.09**	1.16	0.16
C18:0	0.60	0.54	0.54	0.60	0.58	*0.54*	*0.60*	**0.57**	0.03	0.06
19:0 cyc	13.33	13.47	12.30	14.67	13.35	*12.30*	*14.67*	**13.43**	0.84	0.06
C19:0	0.16	0.16	0.14	0.19	<0.01	*<0.01*	*0.19*	**0.13**	0.07	0.57
C20:0	<0.01	<0.01	<0.01	<0.01	<0.01	*<0.01*	*<0.01*			

TABLE 4. RANGE AND AVERAGE VALUES (AREA PERCENT) FOR FAME OF *E. coli* FROM OTTER SAMPLES.

Fatty acid	otter-107	otter-108	otter-109	otter-110	otter-111	otter-112	*MIN*	*MAX*	AVG	SD	RSD
C12:0	2.33	2.92	2.69	3.06	3.24	2.11	*2.11*	*3.24*	**2.72**	0.44	0.16
C13:0	0.08	0.06	0.10	0.08	0.07	0.06	*0.06*	*0.10*	**0.08**	0.01	0.19
3-OH 12:0	0.04	0.03	0.03	0.05	<0.01	0.03	*<0.01*	*0.05*	**0.03**	0.02	0.52
C14:1	0.42	0.41	0.40	0.42	0.56	0.40	*0.40*	*0.56*	**0.44**	0.06	0.15
C14:0	6.24	7.47	6.58	7.36	7.40	6.64	*6.24*	*7.47*	**6.95**	0.52	0.08
UK1	0.76	0.74	0.81	0.78	0.81	0.78	*0.74*	*0.81*	**0.78**	0.03	0.03
ai 15:0	0.03	<0.01	<0.01	<0.01	<0.01	<0.01	*<0.01*	*0.03*	**0.01**	0.01	2.45
C15:0	0.71	0.40	0.64	0.39	0.50	0.51	*0.39*	*0.71*	**0.52**	0.13	0.24
2-OH 14:0	0.28	0.03	0.31	0.23	0.33	0.27	*0.03*	*0.33*	**0.24**	0.11	0.45
3-OH 14:0	6.46	6.45	6.06	6.70	5.89	6.44	*5.89*	*6.70*	**6.33**	0.30	0.05
16:1w7	3.94	4.69	3.33	3.65	4.71	4.91	*3.33*	*4.91*	**4.20**	0.65	0.16
C16:0	31.54	29.77	31.58	30.33	31.39	29.32	*29.32*	*31.58*	**30.65**	0.98	0.03
iso 17:0	0.03	<0.01	<0.01	<0.01	0.06	0.05	*<0.01*	*0.06*	**0.02**	0.03	1.19
C17:1	0.12	<0.01	0.07	<0.01	0.07	0.13	*<0.01*	*0.13*	**0.06**	0.06	0.86
17:0 cyc	22.40	20.08	22.99	21.14	21.78	19.96	*19.96*	*22.99*	**21.39**	1.23	0.06
C17:0	0.32	0.21	0.33	0.22	0.29	0.23	*0.21*	*0.33*	**0.27**	0.05	0.20
2-OH 16.0	0.04	0.11	0.04	0.04	<0.01	0.07	*<0.01*	*0.11*	**0.05**	0.04	0.77
uk2	0.52	0.44	0.41	0.48	0.37	0.31	*0.31*	*0.52*	**0.42**	0.08	0.18
18:1w7	6.74	7.59	5.87	7.22	6.73	7.71	*5.87*	*7.71*	**6.97**	0.68	0.10
C18:0	0.60	0.59	0.61	0.54	0.54	0.59	*0.54*	*0.61*	**0.58**	0.03	0.05
19:0 cyc	11.96	12.92	12.74	13.43	12.05	14.73	*11.96*	*14.73*	**12.97**	1.02	0.08
C19:0	0.16	0.27	0.19	0.12	<0.01	0.23	*<0.01*	*0.27*	**0.16**	0.10	0.60
C20:0	0.06	<0.01	<0.01	<0.01	<0.01	<0.01	*<0.01*	*0.06*	**0.01**	0.02	2.45